FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES **DESIGNATED/ELECTED OFFICE (DO/EO/US)** **CONCERNING A FILING UNDER 35 U.S.C. 371**	**DEX-0109**
	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR **09/700769**

INTERNATIONAL APPLICATION NO. **PCT/US99/10498**	INTERNATIONAL FILING DATE **12 MAY 1999**	PRIORITY DATE CLAIMED **21 MAY 1998**

TITLE OF INVENTION
A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING COLON CANCER

APPLICANT(S) FOR DO/EO/US
MACINA, Roberto A. et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))

    a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).

    b. ☐ has been transmitted by the International Bureau.

    c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ A copy of the International Search Report (PCT/ISA/210).

8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))

    a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).

    b. ☐ have been transmitted by the International Bureau.

    c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.

    d. ☒ have not been made and will not be made.

9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).

11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).

12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

15. ☐ A **FIRST** preliminary amendment.

16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.

17. ☐ A substitute specification.

18. ☐ A change of power of attorney and/or address letter.

19. ☐ Certificate of Mailing by Express Mail

20. ☒ Other items or information:

Verified Statement Claiming Small Entity Status	"Express Mail" Label No. #EL777534840US Date of Deposit – November 20, 2000 I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Box PCT, Washington, D.C. 20231. By _Suzanne Sparkman_ Typed Name: **Suzanne Sparkman**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR **09/700769**	INTERNATIONAL APPLICATION NO. **PCT/US99/10498**	ATTORNEY'S DOCKET NUMBER **DEX-0109**

	CALCULATIONS PTO USE ONLY

21.　　　The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

☐　Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . .　**$1,000.00**

☐　International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO . . . .　**$860.00**

☐　International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . .　**$710.00**

☒　International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) . . . .　**$690.00**

☐　International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) . . . .　**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$690.00**	

Surcharge of **$130.00** for furnishing the oath or declaration later than　☐ 20　☐ 30
months from the earliest claimed priority date (37 CFR 1.492 (e)).　　**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	11　- 20 =	0	x　$18.00	**$0.00**	
Independent claims	5　- 3 =	2	x　$80.00	**$160.00**	
Multiple Dependent Claims (check if applicable).			☒	**$270.00**	
TOTAL OF ABOVE CALCULATIONS　=				**$1,120.00**	

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).**　☒　**$560.00**

SUBTOTAL　=	**$560.00**	

Processing fee of **$130.00** for furnishing the English translation later than　☐ 20　☐ 30
months from the earliest claimed priority date (37 CFR 1.492 (f)).　+　**$0.00**

TOTAL NATIONAL FEE　=	**$560.00**	

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**　☐　**$0.00**

TOTAL FEES ENCLOSED　=	**$560.00**	
	Amount to be: refunded	$
	charged	$

☐　A check in the amount of　　　　　to cover the above fees is enclosed.

☐　Please charge my Deposit Account No.　　　in the amount of　　　to cover the above fees.
　　A duplicate copy of this sheet is enclosed.

☒ **Credit Card Payment form attached.**

☒　The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment

　　to Deposit Account No.　**501-619**　　A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

LICATA, Jane Massey; TYRRELL, Kathleen A.
Licata & Tyrrell P.C.
66 E. Main Street
Marlton, New Jersey　08053　US

SIGNATURE

Jane Massey Licata

NAME

32,257

REGISTRATION NUMBER

20 November 2000

DATE

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) - SMALL BUSINESS CONCERN	Docket No. DEX-0109

Serial No. Not Yet Assigned	Filing Date Herewith	Patent No.	Issue Date

Applicant/ MACINA, Roberto A., et al.
Patentee:

Invention: A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING COLON CANCER

I hereby declare that I am:

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: diaDexus, Inc.

ADDRESS OF CONCERN: 3303 Octavius Drive, Santa Clara, California 95054

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above identified invention described in:

☒ the specification filed herewith with title as listed above.

☐ the application identified above.

☐ the patent identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☒ no such person, concern or organization exists.

☐ each such person, concern or organization is listed below.

FULL NAME _____

ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____

ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____

ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____

ADDRESS _____

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: **Mohan Iyer**

TITLE OF PERSON SIGNING

OTHER THAN OWNER: **Vice President, Business Development**

ADDRESS OF PERSON SIGNING: **diaDexus, Inc.**

3303 Octavius Drive

Santa Clara, California 95054

SIGNATURE: _Mohan Iyer_ DATE: 11/7/00

A NOVEL METHOD OF DIAGNOSING,
MONITORING, AND STAGING COLON CANCER

FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, and prognosticating cancers, particularly colon cancer.

BACKGROUND OF THE INVENTION

Colon cancer is the second most frequently diagnosed malignancy in the United States. Cancer of the gastrointestinal tract, especially colon cancer, is a highly treatable and often a curable disease when localized to the bowel. However, currently colon cancer is the second most common cause of cancer death. Surgery is the primary treatment and results in cure in approximately 50% of patients. Recurrence following surgery is a major problem and often is the ultimate cause of death. The prognosis of colon cancer is clearly related to the degree of penetration of the tumor through the bowel wall and the presence or absence of nodal involvement. These two characteristics form the basis for all staging systems developed for this disease. Bowel obstruction and bowel perforation are indicators of poor prognosis. Elevated pretreatment serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) also have negative prognostic significance.

Because of the frequency of the disease (approximately 160,000 new cases of colon cancer per year), the identification of high-risk groups, the demonstrated slow growth of primary lesions, the better survival of early-stage lesions, and the relative simplicity and accuracy of screening tests, screening for colon cancer should be a part of routine

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care for all adults starting at age 50, especially those with first-degree relatives with colorectal cancer.

Procedures used for detecting, diagnosing, monitoring, staging, and prognosticating colon cancer are of critical importance to the outcome of the patient. For example, patients diagnosed with early colon cancer generally have a much greater five-year survival rate as compared to the survival rate for patients diagnosed with distant metastasized colon cancer. Treatment decisions are usually made in reference to the older Dukes or the Modified Astler-Coller (MAC) classification schema for staging. However, new diagnostic methods which are more sensitive and specific for detecting early colon cancer are clearly needed.

Further, colon cancer patients must be closely monitored following initial therapy and during adjuvant therapy to determine response to therapy and to detect persistent or recurrent disease of metastasis. Thus, there is clearly a need for a colon cancer marker which is more sensitive and specific in detecting colon cancer recurrence.

Another important step in managing colon cancer is to determine the stage of the patient's disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Currently, pathological staging of colon cancer is preferable over clinical staging as pathological staging provides a more accurate prognosis. However, clinical staging would be preferred were the method of clinical staging at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of colon cancer would be improved by detecting new markers in cells, tissues, or bodily fluids which could differentiate between different stages of invasion.

In the present invention, methods are provided for detecting, diagnosing, monitoring, staging, and

prognosticating colon cancers, particularly colon, stomach, and small intestine cancer, via nine (9) Colon Specific Genes (CSGs). The nine CSGs refer, among other things, to native proteins expressed by the genes comprising the polynucleotide sequences of any of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9. In the alternative, what is meant by the nine CSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9 or levels of the genes comprising any of the polynucleotide sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of colon cancer in a patient which comprises measuring levels of CSG in a sample of cells, tissue or bodily fluid from the patient and comparing the measured levels of CSG with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in the measured CSG levels in the patient versus levels of CSG in the control is associated with colon cancer.

Another object of the present invention is to provide a method of diagnosing metastatic colon cancer in a patient which comprises measuring CSG levels in a sample of cells,

tissue, or bodily fluid from the patient and comparing the measured CSG levels with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured CSG levels in the patient versus levels of CSG in the control is associated with a cancer which has metastasized.

Another object of the present invention is to provide a method of staging colon cancer in a patient which comprises identifying a patient having colon cancer, measuring levels of CSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured CSG levels with levels of CSG in preferably the same cells, tissue or bodily fluid type of a control. An increase in measured CSG levels in the patient versus CSG levels in the control can be associated with a cancer which is progressing while a decrease or equivalent level of CSG measured in the patient versus the control can be associated with a cancer which is regressing or in remission.

Another object of the present invention is to provide a method of monitoring colon cancer in a patient for the onset of metastasis. The method comprises identifying a patient having colon cancer that is not known to have metastasized, periodically measuring levels of CSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured CSG levels with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured CSG levels versus control CSG levels is associated with a cancer which has metastasized.

Yet another object of the present invention is to provide a method of monitoring the change in stage of colon cancer in a patient which comprises identifying a patient having colon cancer, periodically measuring levels of CSG in a sample of cells, tissue, or bodily fluid obtained from the patient, and comparing the measured CSG levels with levels of

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CSG in preferably the same cells, tissues, or bodily fluid type of a control wherein an increase in measured CSG levels versus the control CSG levels is associated with a cancer which is progressing and a decrease in the measured CSG levels versus the control CSG levels is associated with a cancer which is regressing or in remission.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging, and prognosticating cancers by comparing levels of CSG with those of CSG in a normal human control. What is meant by "levels of CSG" as used herein, means levels of the native protein expressed by the genes comprising the polynucleotide sequence of any of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9. In the alternative, what is meant by "levels of CSG" as used herein, means levels of the native mRNA encoded by any of the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9 or levels of the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9. Such levels are preferably measured in at least one of, cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for

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diagnosing over-expression of any one of the CSG proteins compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including colon cancer. Any of the nine CSGs may be measured alone in the methods of the invention, or all together or any combination of the nine.

By "control" it is meant a human patient without cancer and/or non cancerous samples from the patient, also referred to herein as a normal human control; in the methods for diagnosing or monitoring for metastasis, control may also include samples from a human patient that is determined by reliable methods to have colon cancer which has not metastasized.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as CSG. Other cancer markers, in addition to CSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of colon cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of CSG in the patient versus the normal human control is associated with the presence of colon cancer. Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic colon cancer in a patient having colon

cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having colon cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art. For example, in the case of colon cancer, patients are typically diagnosed with colon cancer following traditional detection methods.

In the present invention, determining the presence of CSG level in cells, tissues, or bodily fluid, is particularly useful for discriminating between colon cancer which has not metastasized and colon cancer which has metastasized. Existing techniques have difficulty discriminating between colon cancer which has metastasized and colon cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissues, or bodily fluid is CSG, and are compared with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just CSG in serum, this level is preferably compared with the level of CSG in serum of a normal human patient. An increase in the CSG in the patient versus the normal human control is associated with colon cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may also include samples from a human patient that is determined by reliable methods to have colon cancer which has not metastasized.

Staging

The invention also provides a method of staging colon cancer in a human patient.

The method comprises identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG. Then, the method compares CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring colon cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided by this inventions is a method of monitoring the change in stage of colon cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample

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of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CSG is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression, such as CSG of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, in situ hybridization assays, competitive-binding assays, Western Blot analyses and ELISA assays. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.
An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to CSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to CSG is incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific

protein such as bovine serum albumin. Next, the sample to be
analyzed is incubated in the dish, during which time CSG binds
to the specific antibody attached to the polystyrene dish.
Unbound sample is washed out with buffer. A reporter antibody
specifically directed to CSG and linked to horseradish
peroxidase is placed in the dish resulting in binding of the
reporter antibody to any monoclonal antibody bound to CSG.
Unattached reporter antibody is then washed out. Reagents for
peroxidase activity, including a colorimetric substrate are
then added to the dish. Immobilized peroxidase, linked to CSG
antibodies, produces a colored reaction product. The amount
of color developed in a given time period is proportional to
the amount of CSG protein present in the sample. Quantitative
results typically are obtained by reference to a standard
curve.

A competition assay may be employed wherein antibodies
specific to CSG attached to a solid support and labeled CSG
and a sample derived from the host are passed over the solid
support and the amount of label detected attached to the solid
support can be correlated to a quantity of CSG in the sample.
Nucleic acid methods may be used to detect CSG mRNA as a
marker for colon cancer. Polymerase chain reaction (PCR) and
other nucleic acid methods, such as ligase chain reaction
(LCR) and nucleic acid sequence based amplification (NASABA),
can be used to detect malignant cells for diagnosis and
monitoring of various malignancies. For example, reverse-
transcriptase PCR (RT-PCR) is a powerful technique which can
be used to detect the presence of a specific mRNA population
in a complex mixture of thousands of other mRNA species. In
RT-PCR, an mRNA species is first reverse transcribed to
complementary DNA (cDNA) with use of the enzyme reverse
transcriptase; the cDNA is then amplified as in a standard PCR
reaction. RT-PCR can thus reveal by amplification the
presence of a single species of mRNA. Accordingly, if the
mRNA is highly specific for the cell that produces it, RT-PCR

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can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the CSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the CSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.
The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

EXAMPLES

The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments.

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These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

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Example 1: CSGs

Searches were carried out and CSGs identified using the following Search Tools as part of the LIFESEQ® database available from Incyte Pharmaceuticals, Palo Alto, CA:

1.   Library Comparison (compares one library to one other library) allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue.

2.   Subsetting is similar to library comparison but allows the identification of clones expressed in a pool of libraries and absent or expressed at a lower level in a second pool of libraries.

3.   Transcript Imaging lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones can then be examined using Electronic Northerns to determine the tissue sources of their component ESTs.

4.   Protein Function: Incyte has identified subsets of ESTs with a potential protein function based on homologies to known proteins.   Some examples in this database include Transcription Factors and Proteases.   We identified some leads by searching in this database for clones whose component ESTs showed disease specificity.

Electronic subtractions, transcript imaging and protein function searches were used to identify clones, whose component ESTs were exclusively or more frequently found in libraries from specific tumors.   Individual candidate clones were examined in detail by checking where each EST originated.

Table 1: CSGs

SEQ ID NO:	Clone ID #	Gene ID #	
1	238330	242807	Transcript Imaging
2	1285234	239588	Subsetting
3	1341701	29634	Transcript Imaging
4	816257	233421	Subsetting
5	775133	245080	Subsetting
6	1335450	245811	Subsetting
7	2348122	233711	Transcript Imaging
8	3228674	230273	Subsetting
9	1632174	229022	Transcript Imaging

The following example was carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 2: Relative Quantitation of CSG Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

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Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample are used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

To evaluate the tissue distribution, and the level of CSGs in normal and tumor tissue, total RNA was extracted from normal tissues, tumor tissues, and from tumors and the corresponding matched normal tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to the CSG. The results were analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the CSG compared to the calibrator.

Comparative Examples

Similar mRNA expression analysis for genes coding for the diagnostic markers PSA (Prostate Specific Antigen) and PLA2 (Phospholipase A2) was performed for comparison. PSA is currently the only cancer screening marker available in clinical laboratories. When the panel of normal pooled tissues was analyzed, PSA was expressed at very high levels in prostate, with a very low expression in breast and testis. After analysis of more than 55 matching samples from 14 different tissues, the data corroborated the tissue specificity seen with normal tissue samples. PSA expression was compared in cancer and normal adjacent tissue for 12 matching samples of prostate tissue. The relative levels of PSA were higher in 10 cancer samples (83%). Clinical data

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recently obtained support the utilization of PLA2 as a staging marker for late stages of prostate cancer. mRNA expression data described herein showed overexpression of the mRNA in 8 out of the 12 prostate matching samples analyzed (66%). PLA2 had high levels of mRNA expression in small intestine, prostate, liver, and pancreas.

Measurement of SEQ ID NO:3; Clone ID 1341701; Gene ID 29634 (Cln106)

Absolute numbers are depicted in Table 2 as relative levels of expression of Cln106 (SEQ ID NO:3) in 12 normal different tissues. All the values are compared to normal testis (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 2:   Relative levels of Cln106 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	110
Endometrium	0
Kidney	0
Liver	0
Ovary	0
Pancreas	0
Prostate	16
Small Intestine	0
Spleen	0
Stomach	0
Testis	1
Uterus	0

The relative levels of expression in Table 2 show for the CSG Cln106 (SEQ ID NO:3), mRNA expression is more than 6 fold higher in the pool of normal ascending colon (110) compared with prostate (16). Testis, the calibrator, with a relative expression level of 1, is the only other tissue expressing the mRNA for Cln106 (SEQ ID NO:3). These results demonstrate that mRNA expression of this CSG is highly specific for colon.

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The absolute numbers in Table 2 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 3.

The absolute numbers in Table 3 are relative levels of expression of Cln106 (SEQ ID NO:3) in 57 pairs of matching samples. All the values are compared to normal testis (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 3:   Relative levels of Cln106 Expression in Individual Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	4	96
Sto AC99	Stomach 2	0.4	0.5
Sml 21XA	Small Intestine 1	0	0
Sml H89	Small Intestine 2	0.93	1.28
Cln B56	Colon-Cecum(A)1	317	101
Cln AS45	Colon-Ascending(A)2	316.3	146.5
Cln CM67	Colon-Cecum(B)3	481.0	217.5
Cln AS67	Colon-Ascending(B)4	858.1	220.6
Cln AS43	Colon-Ascending(C)5	1370	98
Cln AS46	Colon-Ascending(C)6	3051	375
Cln AS98	Colon-Ascending(C)7	26	42
Cln AS89	Colon-Ascending(D)8	524.6	11.0
Cln TX01	Colon-Transverse(B)9	2886.3	1992.0
Cln TX89	Colon-Transverse(B)10	146.0	35.9
Cln TX67	Colon-Transverse(C)11	2.9	421.7
Cln MT38	Colon-Splenic Flexure(M)12	1681	187

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Cln SG89	Colon-Sigmoid(B)13	1063.8	31.1
Cln SG67	Colon-Sigmoid(C)14	8.5	9.4
Cln SG33	Colon-Sigmoid(C)15	264	549
Cln SG45	Colon-Sigmoid(D)16	580.0	114.6
Cln B34	Colon-Rectosigmoid(A)17	97	244
Cln CXGA	Colon-Rectum(A)18	45.1	273.4
Cln RC67	Colon-Rectum(B)19	2.7	20.0
Cln C9XR	Colon-Rectosigmoid(C)20	609	460
Cln RS45	Colon-Rectosigmoid(C)21	472.8	144.0
Cln RC01	Colon-Rectum(C)22	568	129
Cln RC89	Colon-Rectum(D)23	4.6	322.91
Bld 46XK	Bladder 1	0.2	0
Bld 66X	Bladder 2	1	1
Bld 32XK	Bladder 3	0.0	0.0
Kid 126XD	Kidney 1	0	0
Kid 12XD	Kidney 2	0	0
Kid 5XD	Kidney 3	0.0	1.0
Kid 6XD	Kidney 4	0.0	0.0
Kid 106XD	Kidney 5	0.4	0.0
Liv 42X	Liver 1	0.0	0.0
Liv 15XA	Liver 2	0.0	0.0
Liv 94XA	Liver 3	0.0	0.0
Lng AC69	Lung 1	2	0
Lng BR94	Lung 2	0	0
Lng 47XQ	Lung 3	0	0
Mam 59X	Mammary Gland 1	0	0
Mam B011X	Mammary Gland 2	0	0
Mam A06X	Mammary Gland 3	0	0
Ovr 103X	Ovary 1	0.04	2.08
Ovr 130X	Ovary 2	0.1	2.76

Pan 71XL	Pancreas 1	4.08	0.1
Pan 82XP	Pancreas 2	0	0
Pro 12B	Prostate 1	0.3	0
Pro 23B	Prostate 2	3	4
Pro 13XB	Prostate 3	2	7
Pro 34B	Prostate 4	0.54	4.01
Pro 20XB	Prostate 5	4.8	4.3
Pro 65XB	Prostate 6	0.7	1.3
Tst 39X	Testis 1	2.78	0
End 8XA	Endometrium 1	0	0.2
Utr 85XU	Uterus 1	1.26	0

0= Negative

When matching samples were analyzed, the higher levels of expression were in the colon, showing a high degree of tissue specificity for this tissue. These results confirm the tissue specificity results obtained with the panel of normal pooled samples (Table 2). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 3 shows overexpression of Cln106 (SEQ ID NO:3) in 15 colon cancer tissues compared with their respective normal adjacent (colon samples #1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, 16, 20, 21, and 22). There is overexpression in the cancer tissue for 65% of the colon matching samples tested (total of 23 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in 65% of the colon matching samples

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tested are demonstrative of CSG Cln106 (SEQ ID NO:3) being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:4; Clone ID 816257; Gene ID 406452 (Cln107)

Absolute numbers as depicted in Table 4 are relative levels of expression of CSG Cln107 (SEQ ID NO:4) in 12 normal different tissues. All the values are compared to normal small intestine (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 4:   Relative levels of Cln107 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	3.2
Endometrium	0
Kidney	0.2
Liver	0
Ovary	0
Pancreas	0
Prostate	0.1
Small Intestine	1
Spleen	0
Stomach	0.3
Testis	0
Uterus	0

The relative levels of expression in Table 4 show that mRNA expression of the CSG Cln107 (SEQ ID NO:4) is more than 10 fold higher in the pool of normal ascending colon (3.2), five fold higher in small intestine (1), and 1.5 fold higher in stomach (0.3), compared with the next higher expressor (0.2 for kidney). Seven of the pooled tissues samples analyzed were negative and prostate showed a relative expression of 0.1 for Cln107 (SEQ ID NO:4). These results demonstrate that Cln107 mRNA expression is highly specific for colon, small intestine, and in a lower degree for stomach.

The absolute numbers in Table 4 were obtained analyzing pools of samples of a particular tissue from different

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individuals. They can not be compared to the absolute numbers
originated from RNA obtained from tissue samples of a single
individual in Table 5.

The absolute numbers in Table 5 are relative levels of
expression of Cln107 (SEQ ID NO:4) in 57 pairs of matching
samples. All the values are compared to normal small
intestine (calibrator). A matching pair is formed by mRNA
from the cancer sample for a particular tissue and mRNA from
the normal adjacent sample for that same tissue from the same
individual.

Table 5:   **Relative levels of Cln107 Expression in Individual**
             Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	8.9	13.4
Sto AC99	Stomach 2	6.0	0.9
Sml 21XA	Small Intestine 1	1.07	1.42
Sml H89	Small Intestine 2	0.97	4.13
Cln B56	Colon-Cecum(A)1	2	16
Cln AS45	Colon-Ascending(A)2	0.7	2.1
Cln CM67	Colon-Cecum(B)3	1.6	2.1
Cln AS67	Colon-Ascending(B)4	1.2	6.2
Cln AS43	Colon-Ascending(C)5	13.5	0.5
Cln AS46	Colon-Ascending(C)6	9.7	23.6
Cln AS98	Colon-Ascending(C)7	28.1	1.4
Cln AS89	Colon-Ascending(D)8	0.9	3.1
Cln TX01	Colon-Transverse(B)9	3.0	10.6
Cln TX89	Colon-Transverse(B)10	4.5	0.6
Cln TX67	Colon-Transverse(C)11	3.6	3.4
Cln MT38	Colon-Splenic Flexture(M)12	4.0	2.6
Cln SG89	Colon-Sigmoid(B)13	4.7	0.9

Cln SG67	Colon-Sigmoid(C)14	1.0	1.3
Cln SG33	Colon-Sigmoid(C)15	14.2	7.6
Cln SG45	Colon-Sigmoid(D)16	4.8	6.0
Cln B34	Colon-Rectosigmoid(A)17	3	2
Cln CXGA	Colon-Rectum(A)18	4.4	1.9
Cln RC67	Colon-Rectum(B)19	0.1	0.4
Cln C9XR	Colon-Rectosigmoid(C)20	5	3
Cln RS45	Colon-Rectosigmoid(C)21	11.4	4.6
Cln RC01	Colon-Rectum(C)22	1.8	2.3
Cln RC89	Colon-Rectum(D)23	0.1	5.35
Bld 46XK	Bladder 1	0.2	0
Bld 66X	Bladder 2	1	1
Bld 32XK	Bladder 3	0.1	0.1
Kid 126XD	Kidney 1	0	0.02
Kid 12XD	Kidney 2	0.1	0.2
Kid 5XD	Kidney 3	0.3	0.0
Kid 6XD	Kidney 4	0.1	0.1
Kid 106XD	Kidney 5	0.0	0.1
Liv 42X	Liver 1	7.9	0.002
Liv 15XA	Liver 2	0.0	0.0
Liv 94XA	Liver 3	0.0	0.0
Lng AC69	Lung 1	1.6	0.2
Lng BR94	Lung 2	0.4	0
Lng 47XQ	Lung 3	0.78	0.2
Mam 59X	Mammary Gland 1	0.05	0.3
Mam B011X	Mammary Gland 2	0.01	0.004
Mam A06X	Mammary Gland 3	0.22	0
Ovr 103X	Ovary 1	0.01	0.01
Ovr 130X	Ovary 2	0.09	0.1
Pan 71XL	Pancreas 1	2.51	2.81

Pan 82XP	Pancreas 2	0	0.62
Pro 12B	Prostate 1	0.3	0.1
Pro 23B	Prostate 2	0.3	0.2
Pro 13XB	Prostate 3	0	0
Pro 34B	Prostate 4	0.04	0.22
Pro 20XB	Prostate 5	0.4	0.1
Pro 65XB	Prostate 6	0.0	0.1
Tst 39X	Testis 1	0.02	0.01
End 8XA	Endometrium 1	0.01	0.5
Utr 85XU	Uterus 1	0.03	0

0= Negative

When matching samples were analyzed, the higher levels
of expression were in colon, stomach, and small intestine,
showing a high degree of tissue specificity for colon tissues.
These results confirm the tissue specificity results obtained
with normal pooled samples (Table 4). Furthermore, the level
of mRNA expression in cancer samples and the isogenic normal
adjacent tissue from the same individual were compared. This
comparison provides an indication of specificity for the
cancer stage (e.g. higher levels of mRNA expression in the
cancer sample compared to the normal adjacent). Table 5 shows
overexpression of Cln107 (SEQ ID NO:4) in 11 colon cancer
tissues compared with their respective normal adjacent (colon
samples #5, 7, 10, 11, 12, 13, 15, 17, 18, 20, and 21). There
is overexpression in the cancer tissue for 48% of the colon
matching samples tested (total of 23 colon matching samples).
The matching sample Pan 71XL is a secondary cancer in
pancreas, the primary cancer in that individual was a duodenal
cancer.

Altogether, the high level of tissue specificity, plus
the mRNA overexpression in almost half of the colon, stomach,
and small intestine matching samples tested are demonstrative

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of CSG Cln107 (SEQ ID NO:4) being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:5; Clone ID 775133; Gene ID 24508 (Cln108)

The absolute numbers shown in Table 6 are relative levels of expression of CSG Cln108 (SEQ ID NO:5) in 12 normal different tissues. All the values are compared to normal small intestine (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 6:   Relative levels of Cln108 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	2846.5
Endometrium	1
Kidney	5.5
Liver	18.7
Ovary	3.4
Pancreas	198.1
Prostate	1024
Small Intestine	810.8
Spleen	32.2
Stomach	9981.2
Testis	0
Uterus	294.1

The relative levels of expression in Table 6 show that mRNA expression of CSG Cln108 (SEQ ID NO:5) is more than 10 fold higher in the pool of normal ascending colon (2846.5) and almost ten fold higher in stomach (9981.2), compared to the expression level in any other tissue analyzed. These results demonstrate that mRNA expression of this CSG is also highly specific for colon and stomach.

The absolute numbers in Table 6 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 7.

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The absolute numbers depicted in Table 7 are relative levels of expression of Cln108 (SEQ ID NO:5) in 57 pairs of matching samples. All the values are compared to normal small intestine (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 7:   Relative levels of Cln108 Expression in Individual Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	28696	34842
Sto AC99	Stomach 2	21523	30862
Sml 21XA	Small Intestine 1	2944	964.4
Sml H89	Small Intestine 2	244.5	3513.2
Cln B56	Colon-Cecum(A)1	27242	24637
Cln AS45	Colon-Ascending(A)2	5827.0	8771.0
Cln CM67	Colon-Cecum(B)3	4251.0	4684.0
Cln AS67	Colon-Ascending(B)4	564.0	1949.0
Cln AS43	Colon-Ascending(C)5	50310	10949
Cln AS46	Colon-Ascending(C)6	246044	120073
Cln AS98	Colon-Ascending(C)7	40442	17482
Cln AS89	Colon-Ascending(D)8	5730.0	1581.0
Cln TX01	Colon-Transverse(B)9	22281.0	114784.0
Cln TX89	Colon-Transverse(B)10	11026.0	1639.0
Cln TX67	Colon-Transverse(C)11	17004.0	11654.0
Cln MT38	Colon-Splenic Flexture(M)12	77589	31620
Cln SG89	Colon-Sigmoid(B)13	140339.0	49617.0
Cln SG67	Colon-Sigmoid(C)14	4951.0	7905.0
Cln SG33	Colon-Sigmoid(C)15	60875	120490

Cln SG45	Colon-Sigmoid(D)16	30437.0	47267.0
Cln B34	Colon-Rectosigmoid(A)17	5848	5861
Cln CXGA	Colon-Rectum(A)18	13877.0	9787.0
Cln RC67	Colon-Rectum(B)19	1703.0	26589.0
Cln C9XR	Colon-Rectosigmoid(C)20	2458	19071
Cln RS45	Colon-Rectosigmoid(C)21	95523	61939
Cln RC01	Colon-Rectum(C)22	98891.0	80047.0
Cln RC89	Colon-Rectum(D)23	17.0	1775
Bld 46XK	Bladder 1	0	8
Bld 66X	Bladder 2	397	44
Bld 32XK	Bladder 3	0.0	16.0
Kid 126XD	Kidney 1	32	22
Kid 12XD	Kidney 2	6	0
Kid 106XD	Kidney 3	4.0	33.0
Liv 42X	Liver 1	4783	0
Liv 15XA	Liver 2	4.0	10.0
Liv 94XA	Liver 3	159.0	21.0
Lng AC69	Lung 1	222	295
Lng BR94	Lung 2	112	0
Lng 47XQ	Lung 3	30	69
Lng AC66	Lung 4	29	137
Mam 59X	Mammary Gland 1	56	0
Mam B011X	Mammary Gland 2	54	31
Mam A06X	Mammary Gland 3	12	0
Ovr 103X	Ovary 1	37	0
Pan 71XL	Pancreas 1	13203	4163
Pan 82XP	Pancreas 2	39.1	0
Pro 12B	Prostate 1	386	88
Pro 23B	Prostate 2	250	23
Pro 13XB	Prostate 3	92	731

Pro 34B	Prostate 4	33.3	265.7
Pro 20XB	Prostate 5	454.6	1908.9
Pro 65XB	Prostate 6	733.5	922.0
End 8XA	Endometrium 1	5	92
Utr 85XU	Uterus 1	98.9	21.8
Utr 23XU	Uterus 2	35.3	0
Utr 135XO	Uterus 3	39.2	43.8
Utr 141XO	Uterus 4	212.1	55.9

0= Negative

When matching samples were analyzed, the higher levels of expression were in colon and stomach, showing a high degree of tissue specificity for these two tissues. These results confirm the tissue specificity results obtained with normal pooled samples (Table 6). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 7 shows overexpression of CSG Cln108 (SEQ ID NO:5) in 13 colon cancer tissues compared with their respective normal adjacent (colon samples #1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 18, 21, and 22). There is overexpression in the cancer tissue for 56% of the colon matching samples tested (total of 23 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in more than half of the colon, stomach, and small intestine matching samples tested are demonstrative of this CSG, Cln108 (SEQ ID NO:5), also being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:7;   Clone ID 2348122; Gene ID 23371 (Cln109)

The absolute numbers depicted in Table 8 are relative levels of expression of CSG Cln109 (SEQ ID NO:7) in 12 normal different tissues.   All the values are compared to normal ovary (calibrator).   These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 8:   Relative levels of Cln109 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	28.8
Endometrium	0.45
Kidney	0.41
Liver	0.72
Ovary	0.07
Pancreas	82.8
Prostate	124.3
Small Intestine	626.4
Spleen	1.2
Stomach	12.05
Testis	1.51
Uterus	52.99

The relative levels of expression in Table 8 show that mRNA expression of CSG Cln109 (SEQ ID NO:7), is more than 5 fold higher in the pool of normal small intestine (626.4) compared to the expression level in any other tissue analyzed.   These results demonstrate that Cln109 (SEQ ID NO:7) mRNA expression is highly specific for small intestine

The absolute numbers in Table 8 were obtained analyzing pools of samples of a particular tissue from different individuals.   They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 9.

The absolute numbers depicted in Table 9 are relative levels of expression of Cln109 (SEQ ID NO:7) in 53 pairs of matching samples.   All the values are compared to normal ovary (calibrator).   A matching pair is formed by mRNA from the

cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 9:   **Relative levels of Cln109 Expression in Individual Samples**

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	2574	1310
Sto AC99	Stomach 2	4153	5
Sml 21XA	Small Intestine 1	2667	13663.8
Sml H89	Small Intestine 2	57.8	904.29
Cln B56	Colon-Cecum(A)1	6794	299
Cln AS45	Colon-Ascending(A)2	814.6	105.8
Cln CM67	Colon-Cecum(B)3	294.6	36.1
Cln AS67	Colon-Ascending(B)4	2.2	26.3
Cln AS43	Colon-Ascending(C)5	111	377
Cln AS46	Colon-Ascending(C)6	1180	352
Cln AS98	Colon-Ascending(C)7	1075	92
Cln AS89	Colon-Ascending(D)8	14022.7	87.5
Cln TX01	Colon-Transverse(B)9	1027.6	282.1
Cln TX89	Colon-Transverse(B)10	2.5	23.7
Cln TX67	Colon-Transverse(C)11	0.1	72.3
Cln MT38	Colon-Splenic Flexture(M)12	372	88
Cln SG89	Colon-Sigmoid(B)13	179.2	33.4
Cln SG67	Colon-Sigmoid(C)14	85.0	94.7
Cln SG33	Colon-Sigmoid(C)15	5461	377
Cln SG45	Colon-Sigmoid(D)16	762.7	15.9
Cln B34	Colon-Rectosigmoid(A)17	460	1
Cln RC67	Colon-Rectum(B)18	64.5	136.2
Cln C9XR	Colon-Rectosigmoid(C)19	441	34
Cln RS45	Colon-Rectosigmoid(C)20	1931	195

Cln RC01	Colon-Rectum(C)21	72.8	19.1
Cln RC89	Colon-Rectum(D)22	4.8	90.2
Bld 46XK	Bladder 1	4	3
Bld 66X	Bladder 2	1	0
Bld 32XK	Bladder 3	0.1	307.6
Kid 126XD	Kidney 1	0	2
Kid 12XD	Kidney 2	3	16
Kid 5XD	Kidney 3	0.0	0.3
Kid 6XD	Kidney 4	18.5	1.2
Liv 42X	Liver 1	21	0.03
Liv 15XA	Liver 2	0.5	0.4
Liv 94XA	Liver 3	0.4	0.0
Lng AC69	Lung 1	0.1	0
Lng BR94	Lung 2	3	0
Lng 60XL	Lung 3	0.1	0
Mam 59X	Mammary Gland 1	0	4
Mam B011X	Mammary Gland 2	8	13
Mam A06X	Mammary Gland 3	4.7	9.6
Pan 71XL	Pancreas 1	8902.5	1428.2
Pan 82XP	Pancreas 2	0.2	9.3
Pro 12B	Prostate 1	9	20
Pro 23B	Prostate 2	191	88
Pro 13XB	Prostate 3	12	460
Pro 34B	Prostate 4	3.2	80.4
Tst 39X	Testis 1	29.9	0
End 8XA	Endometrium 1	0.3	21
Utr 85XU	Uterus 1	244.7	592.2
Ovr 63A	Ovary 1	11.4	0
Ovr A1C	Ovary 2	68.4	0

0= Negative

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When matching samples were analyzed, the higher levels of expression were in small intestine, colon and stomach, showing a high degree of tissue specificity for these three colon tissues. These results confirm the tissue specificity results obtained with normal pooled samples for small intestine (Table 8). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 9 shows overexpression of CSG, Cln109 (SEQ ID NO:7) in 15 colon cancer tissues compared with their respective normal adjacent (colon samples #1, 2, 3, 6, 7, 8, 9, 12, 13, 15, 16, 17, 19, 20, and 21). There is overexpression in the cancer tissue for 68% of the colon matching samples tested (total of 22 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in more than half of the colon, stomach, and small intestine matching samples tested are demonstrative of CSG Cln109 (SEQ ID NO:7) being a diagnostic marker for colon cancer. The amino acid sequence encoded by the open reading frame of Cln109 is depicted in SEQ ID NO:10.

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What is claimed is:

1. A method for diagnosing the presence of colon cancer in a patient comprising:

(a) measuring levels of CSG in a sample of cells, tissue or bodily fluid obtained from the patient; and

(b) comparing the measured levels of CSG with levels of CSG in a sample of cells, tissue or bodily fluid obtained from a control, wherein an increase in measured levels of CSG in the patient versus the CSG levels in the control is associated with the presence of colon cancer.

2. A method of diagnosing metastatic colon cancer in a patient comprising:

(a) measuring levels of CSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and

(b) comparing the measured levels of CSG with levels of CSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in measured CSG levels in the patient versus the CSG levels in the control is associated with a cancer which has metastasized.

3. A method of staging colon cancer in a patient comprising:

(a) identifying a patient suffering from colon cancer;

(b) measuring levels of CSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of CSG with levels of CSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in the measured levels of CSG versus the levels of CSG in the control is associated with a cancer which is progressing and a decrease in the measured levels of CSG versus the levels of CSG in the control is associated with a cancer which is regressing or in remission.

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4.   A method of monitoring colon cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having colon cancer that is not known to have metastasized;

(b) periodically measuring CSG levels in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the periodically measured levels of CSG with levels of CSG in cells, tissue, or bodily fluid obtained from a control, wherein an increase in any one of the periodically measured levels of CSG in the patient versus the levels of CSG in the control is associated with a cancer which has metastasized.

5.   A method of monitoring changes in a stage of colon cancer in a patient comprising:

(a) identifying a patient having colon cancer;

(b) periodically measuring levels of CSG in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of CSG with levels of CSG in a sample of the same cells, tissue, or bodily fluid of a control, wherein an increase in any one of the periodically measured levels of CSG versus levels of CSG in the control is associated with a cancer which is progressing in stage and a decrease in any one of the periodically measured levels of CSG versus the levels of CSG in the control is associated with a cancer which is regressing in stage or in remission.

6.   The method of claim 1, 2, 3, 4 or 5 wherein the CSG comprises SEQ ID NO:3, 4, 5 or 7.

SEQUENCE LISTING

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<213> Homo sapiens

<400> 10

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Gly Phe Ser Pro Phe Pro Gly Val Asp Ser Ser Ser Ser Phe Ser Ser
        35              40              45

Ser Ser Arg Ser Gly Ser Ser Ser Ser Arg Ser Leu Gly Ser Gly Gly
    50              55              60

Ser Val Ser Gln Leu Phe Ser Asn Phe Thr Gly Ser Val Asp Asp Arg
65              70              75              80

Gly Thr Cys Gln Cys Ser Val Ser Leu Pro Asp Thr Thr Phe Pro Val
            85              90              95

Asp Arg Val Glu Arg Leu Glu Phe Thr Ala His Val Leu Ser Gln Lys
            100             105             110

Phe Glu Lys Glu Leu Ser Lys Val Arg Glu Tyr Val Gln Leu Ile Ser
        115             120             125

Val Tyr Glu Lys Lys Leu Leu Asn Leu Thr Val Arg Ile Asp Ile Met
    130             135             140

Glu Lys Asp Thr Ile Ser Tyr Thr Glu Leu Asp Phe Glu Leu Ile Lys
145             150             155             160

Val Glu Val Lys Glu Met Glu Lys Leu Val Ile Gln Leu Lys Glu Ser
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Phe Gly Gly Ser Ser Glu Ile Val Asp Gln Leu Glu Val Glu Ile Arg
            180             185             190

Asn Met Thr Leu Leu Val Glu Lys Leu Glu Thr Leu Asp Lys Asn Asn
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Pro Ser Val Val Gln Leu Asn Trp Arg Gly Phe Ser Tyr Leu Tyr Gly
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Ala Trp Gly Arg Asp Tyr Ser Pro Gln His Pro Asn Lys Gly Leu Tyr
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              290   295   300

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              325   330   335

Asn Met Tyr Val Asn Met Tyr Asn Thr Gly Asn Ile Ala Arg Val Asn
              340   345   350

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Tyr Asn Asn Arg Phe Ser Tyr Ala Asn Val Ala Trp Gln Asp Ile Asp
              370   375   380

Phe Ala Val Asp Glu Asn Gly Leu Trp Val Ile Tyr Ser Thr Glu Ala
385   390   395   400

Ser Thr Gly Asn Met Val Ile Ser Lys Leu Asn Asp Thr Thr Leu Gln
              405   410   415

Val Leu Asn Thr Trp Tyr Thr Lys Gln Tyr Lys Pro Ser Ala Ser Asn
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Ala Phe Met Val Cys Gly Val Leu Tyr Ala Thr Arg Thr Met Asn Thr
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Gly Lys Leu Asp Ile Val Met His Lys Met Gln Glu Lys Val Gln Ser
465   470   475   480

Ile Asn Tyr Asn Pro Phe Asp Gln Lys Leu Tyr Val Tyr Asn Asp Gly
              485   490   495

Tyr Leu Leu Asn Tyr Asp Leu Ser Val Leu Gln Lys Pro Gln
          500   505   510

09/ 09

| Docket No. |
| DEX-0109 |

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING COLON CANCER

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on   **12 May 1999**   as United States Application No. or PCT International

Application Number   **PCT/US99/10498**

and was amended on

                                    (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)   Priority Not Claimed

			☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/086,266   **21 May 1998**

(Application Serial No.)   (Filing Date)

(Application Serial No.)   (Filing Date)

(Application Serial No.)   (Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Docket No.
DEX-0109

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

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the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **12 May 1999** as United States Application No. or PCT International Application Number **PCT/US99/10498**

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

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Prior Foreign Application(s)   Priority Not Claimed

			☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	

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(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

26259
PATENT _TRADEMARK OFFICE

Send Correspondence to:

Direct Telephone Calls to: *(name and telephone number)*
Jane Massey Licata or Kathleen A. Tyrrell - (856) 810-1515

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Sole or first inventor's signature	Date
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Citizenship **US**	
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Second inventor's signature	Date 12/30/2000
Residence **San Diego, California**	
Citizenship **China**	
Post Office Address **12227 Branicole Lane**	
San Diego, California	

Full name of third inventor, if any
Yongming Sun

Third inventor's signature	Date

Residence
San Jose, California

Citizenship
US

Post Office Address
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San Jose, California

Full name of fourth inventor, if any

Fourth inventor's signature	Date

Residence

Citizenship

Post Office Address

Full name of fifth inventor, if any

Fifth inventor's signature	Date

Residence

Citizenship

Post Office Address

Full name of sixth inventor, if any

Sixth inventor's signature	Date

Residence

Citizenship

Post Office Address

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

26259

PATENT _TRADEMARK OFFICE

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Full name of sole or first inventor Roberto A. Macina	
Sole or first inventor's signature	Date 11/7/00
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San Jose, California	

Full name of second inventor, if any Fei Yang	
Second inventor's signature	Date
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Citizenship US	
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San Diego, California	

Full name of third inventor, if any
Yongming Sun

Third inventor's signature

Date
11-6-2000

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Citizenship
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San Jose, California

Full name of fourth inventor, if any

Fourth inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of fifth inventor, if any

Fifth inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of sixth inventor, if any

Sixth inventor's signature

Date

Residence

Citizenship

Post Office Address

Form PTO-SB-01 (6-95) (Modified)

Patent and Trademark Office-U.S. DEPARTMENT OF COMMERCE